

WHAT IS CLAIMED IS:

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1. A modified enzyme wherein at least one acidic amino acid is replaced with at least one neutral amino acid and wherein the basic amino acids at the coenzyme binding site of said enzyme are not replaced; wherein the modified enzyme exhibits increased NAD(H) affinity compared to an unmodified enzyme.
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2. The modified enzyme of claim 1, which is a dehydrogenase enzyme.
3. The modified enzyme of claim 2, which is an alcohol dehydrogenase enzyme.
4. The modified enzyme of Claim 2, which is a rec-(R)-alcohol dehydrogenase enzyme.
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5. The modified enzyme of claim 4, which is a *L. brevis* or *L. kefir* rec-(R)-alcohol dehydrogenase enzyme.
6. The modified enzyme of claim 1, which comprises the amino acid sequence of SEQ ID NO:2.
- Sul C2*
7. The modified enzyme of claim 5, which is a *L. brevis* rec-(R)-alcohol dehydrogenase and which comprises a Glycine to Aspartic Acid amino acid change at amino acid 38.
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8. An isolated polynucleotide which encodes the modified enzyme of claim 1.
9. The isolated polynucleotide of claim 8, which comprises the nucleotide sequence of SEQ ID NO:1.
10. A plasmid vector comprising the isolated polynucleotide of claim 8.
11. A host cell comprising the isolated polynucleotide of claim 8.
- Sul C3*
12. A method of modifying an enzyme comprising: replacing at least one neutral

amino acid in said enzyme with at least one acidic amino acid, wherein the basic amino acids at the coenzyme binding site of said enzyme are not replaced; and wherein said modified enzyme exhibits increased NAD(H) affinity compared to an unmodified enzyme.

- 5        13. The method of claim 12, wherein said enzyme is a dehydrogenase enzyme.
- 10      14. The method of claim 13, wherein said enzyme is an alcohol dehydrogenase enzyme.
- 15      15. The method of Claim 13, wherein said enzyme is a rec-(R)-alcohol dehydrogenase enzyme.
- 20      16. The method of claim 15, wherein said enzyme is a *L. brevis* or *L. kefir* rec-(R)-alcohol dehydrogenase enzyme.
- 25      17. The method of claim 12, wherein said enzyme comprises the amino acid sequence of SEQ ID NO:2.
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- 15 *bul C4*      18. The method of claim 12, which is a *L. brevis* rec-(R)-alcohol dehydrogenase and which comprises a Glycine to Aspartic Acid amino acid change at amino acid 38.
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- 20      19. A method of making the modified enzyme which has improved NAD(H) affinity comprising culturing the cell of claim 8 for a time and under conditions suitable for the expression of the polynucleotide which encodes said enzyme; and collecting the enzyme.
- 25      20. The isolated nucleotide sequences of SEQ ID NO:4 and SEQ ID NO:5.
- 30      21. Sense and antisense polynucleotides which encode TDRHSDVG. *not identical*
- 35      22. A method of enantioselective reduction of a organic compound comprising reacting said compound with the enzyme of claim 1 and at least one of

NAD(H) and NAD<sup>+</sup>, wherein said organic compound is selected from the group selected from the group consisting of ketones,  $\alpha$ -keto esters,  $\beta$ -keto esters,  $\gamma$ -keto esters, and combinations thereof.

23. The method of claim 22, which yields a chiral alcohol.
24. The method of claim 23, wherein said chiral alcohol is an (R)-alcohol.
25. The method of claim 22, wherein said reacting is with NAD(H).
26. The method of claim 22, wherein said enzyme is a dehydrogenase enzyme.
27. The method of claim 22, wherein said enzyme an alcohol dehydrogenase enzyme.
28. The method of claim 22, wherein said enzyme is a rec-(R)-alcohol dehydrogenase enzyme.
29. The method of claim 22, wherein said enzyme is a *L. brevis* or *L. kefir* rec-(R)-alcohol dehydrogenase enzyme.
30. The method of claim 22, wherein said enzyme comprises the amino acid sequence of SEQ ID NO:2.
31. The method of claim 22, wherein said enzyme is a *L. brevis* rec-(R)-alcohol dehydrogenase and which comprises a Glycine to Aspartic Acid amino acid change at amino acid 38.
32. A method of enantioselective oxidation of alcohols comprising reacting an alcohol comprising reacting a alcohol with the enzyme of claim 1 and at least one of NAD(H) and NAD<sup>+</sup>.
33. The method of claim 32, which yields a chiral alcohol.
34. The method of claim 33, wherein said chiral alcohol is a (R)-alcohol.
35. The method of claim 32, wherein said reacting is with NAD(H).

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- 36. The method of claim 32, wherein said enzyme is a dehydrogenase enzyme.
  - 37. The method of claim 32, wherein said enzyme an alcohol dehydrogenase enzyme.
  - 38. The method of claim 32, wherein said enzyme is a rec-(R)-alcohol dehydrogenase enzyme.
  - 39. The method of claim 32, wherein said enzyme is a *L. brevis* or *L. kefir* rec-(R)-alcohol dehydrogenase enzyme.
  - 40. The method of claim 32, wherein said enzyme comprises the amino acid sequence of SEQ ID NO:2.
  - 41. The method of claim 32, wherein said enzyme is a *L. brevis* rec-(R)-alcohol dehydrogenase and which comprises a Glycine to Aspartic Acid amino acid change at amino acid 38.